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Tossed salads

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The Yazidi are members of an ancient Kurdish religion that incorporates elements of Zoroastrianism, Christianity and Islam. Yazidis are forbidden from eating lettuce.[1] A number of explanations have been offered for the sect's aversion to this particular vegetable, including the mob-pelting of a 13th Century Yazidi saint's corpse with heads of lettuce. In the absence of evidence to the contrary, however, I am going to offer the more prosaic explanation that a sane "public health expert" banned the vegetable after linking it to a severe gastroenteritis outbreak.

Closer to home, the FDA warned consumers against eating spinach tainted with enterohemorrhagic *Escherichia coli* in 2006, and against consuming Serrano peppers contaminated with Salmonella in 2008. Contaminated produce was responsible for 2% of all foodborne disease outbreaks in the 1970–1980s, and this rose to 6% in the 1990s; data from 2004 suggests that the incidence could now be as high as 13% of all outbreaks.[2] Since a lot of produce is consumed uncooked, extensive washing of fruits and vegetables is the only practical method of control at the level of the consumer. This, however, may not be effective since foodborne pathogens appear to form intimate associations with plants, often localizing deep within the plant tissues.

Many bacteria find their way into plants through stomata, the microscopic pores on leaf surfaces. Responding to a variety of environmental cues, two specialized epidermal cells known as guard cells regulate the opening and closing of stomata to facilitate photosynthetic gas exchange and regulate water loss. For example, the plant hormone abscisic acid and the guard cell kinase OST1 mediate stomatal closure in response to water limitation. Guard cells also respond to microbe-associated molecular patterns (MAMPS) such as lipopolysaccharide and flagellin, and induce stomatal closure[3]. Thus, stomata play a role in innate immunity by preventing bacterial entry. While the LPS receptor remains uncharacterized, flagellin is sensed by FLS2, a receptor that contains a leucine-rich repeat (LRR) domain and an intracellular serine/threonine kinase domain, and displays homology to toll-like receptors (TLRs). Receptor engagement leads to the activation of reactive oxygen species and MAP kinase 3 (MPK3), leading to stomatal closure.

Many plant pathogens have evolved the ability to enter leaves by specifically triggering the reopening of stomata. For instance, inoculation of the thale cress plant, *Arabidopsis thaliana,* with the plant pathogen *Pseudomonas syringae* causes rapid closure of stomata (within 3 hours).[4] *P. syringae,* however, produces coronatine, a small molecule that mimics some of the properties of the plant hormone methyl jasmonate and triggers the reopening of stomata. *P. syringae* inoculated on leaf surfaces actively move towards open stomata and into the leaf interior, and form microcolonies in close association with the photosynthesizing mesophyll cells. *P. syringae* defective for coronatine production are unable to cause disease when inoculated on the leaf surface, but are virulent when injected directly into the leaves. Stomata of *ost1* mutants of *A. thaliana* remain open in the presence of flagellar peptides, and these plants are equally susceptible to wild-type and coronatine-deficient *P. syringae.*

A somewhat surprising discovery was that *Salmonella* Typhimurium inoculated

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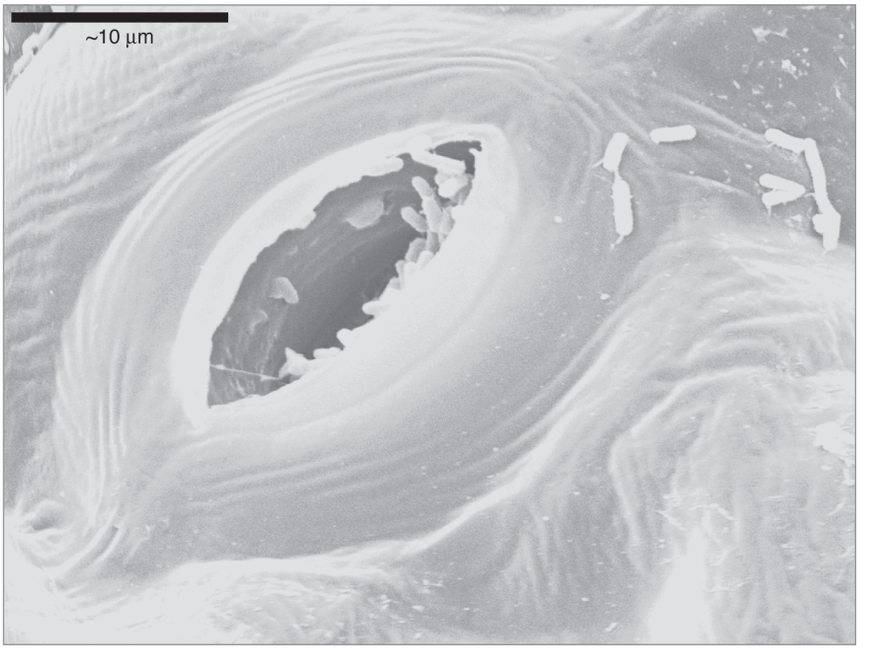

Figure 1. *Salmonella* slips in. Scanning electron microscopy image shows *Salmonella* migrating to, and settling within, the stomatal opening. Image kindly provided by Shlomo Sela, Agricultural Research Organization, The Volcani Center, Beth-Dagan, Israel.

on iceberg lettuce leaves was less effective than *P. syringae* in causing stomatal closure.[5] Moreover, the inoculated bacteria migrated towards open stomata and entered the interior of the leaf (**Fig. 1**)—but only in the presence of light! Attracted by the fresh aroma of photosynthesizing mesophill cells, the bacteria rev their flagella into action and migrate towards the open stomatal window and find their way inside. *S. enterica* evinced no interest in stomata forcibly opened in the dark by the fungal compound fusicocin. Flagellar mutants fail to make the journey, and abolishing the chemotactic gradient by externally applying various nutrients (including leaf extracts) prevented wild-type bacterial migration to the stomata.

These are strategies worthy of a bonafide plant pathogen. Indeed, an earlier study presented evidence to suggest that *Salmonella typhimurium* may cause infections in plants.[6] *S. typhimurium* infects different *Arabidopsis* tissues, proliferates in intracellular compartments, and causes disease symptoms such as wilting and death of infected regions. Arabidopsis mounts a typical innate immune response to Salmonella infection by activating MAP kinases and pathogenesis-related genes. The plants were evidently responding to multiple Salmonella-associated molecules including flagellin. It is curious, then, that these MAMPS were unable to trigger stomatal closure in lettuce leaves. The role of known Salmonella virulence factors including the type III secretion system and associated effectors, if any, in plant pathogenesis remains to be determined.

Unlike Salmonella and *P. syringae,* enterohemorrhagic *E. coli* (EHEC O157:H7) caused stomatal closure and was unable to induce its subsequent opening. EHEC is known to interact closely with spinach leaves in a flagellin- and type III secretion system-dependent manner, but does not replicate effectively within the leaf tissue. The interactions are thus quite variable, and we need to understand them better so that we can devise effective methods to prevent these pathogens from getting into our salad bowls. Failing this, of course, we will have to shun lettuce, not to mention tomatoes and Serrano peppers, like the Yazidis.

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